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Sleep electroencephalogram delta-frequency amplitude, night plasma levels of tumor necrosis factor α, and human immunodeficiency virus infection

D. F. DARKO*, JAMES C. MILLER*, CHRISTOPHER GALLEN*, JEANNINE WHITE†, JAMES KOZIOL*, STEPHEN J. BROWN‡, ROZA HAYDUK†, J. HAMPTON ATKINSON‡, JOSEPH ASSMUS†, DEAN T. MUNNELL‡, PAUL NAITOH§, J. ALLEN MCCUTCHAN‡, AND MERRILL M. MITLER*

*Departments of Neuropharmacology and Molecular Biology, The Committee for Sleep Disorders Research, The Scripps Research Institute, La Jolla, CA 92037; †Department of Medicine, Scripps Clinic and Research Foundation, La Jolla, CA 92037; ‡University of California, San Diego, School of Medicine, La Jolla, CA 92093; and §Naval Health Research Center, San Diego, CA 92186

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ABSTRACT **We tested the hypothesis that increases in tumor necrosis factor α (TNF-α) induced by human immunodeficiency virus (HIV) are associated with the increases in slow-wave sleep seen in early HIV infection and the decrease with sleep fragmentation seen in advanced HIV infection. Nocturnal sleep disturbances and associated fatigue contribute to the disability of HIV infection. TNF-α causes fatigue in clinical use and promotes slow-wave sleep in animal models. With slow progress toward a vaccine and weak effects from current therapies, efforts are directed toward extending productive life of HIV-infected individuals and shortening the duration of disability in terminal illness. We describe previously unrecognized nocturnal cyclic variations in plasma levels of TNF-α in all subjects. In 6 of 10 subjects (1 control subject, 3 HIV-seropositive patients with $CD4^+$ cell number >400 cells per μl, and 2 HIV-positive patients with $CD4^+$ cell number <400 cells per μl), these fluctuations in TNF-α were coupled to the known rhythm of electroencephalogram delta amplitude (square root of power) during sleep. This coupling was not present in 3 HIV-positive subjects with $CD4^+$ cell number <400 cells per μl and 1 control subject. In 5 HIV subjects with abnormally low $CD4^+$ cell counts (<400 cells per μl), the number of days since seroconversion correlated significantly with low correlation between TNF-α and delta amplitude. We conclude that a previously unrecognized normal, physiological coupling exists between TNF-α and delta amplitude during sleep and that the lessened likelihood of this coupling in progressive HIV infection may be important in understanding fatigue-related symptoms and disabilities.**

Fatigue in human immunodeficiency virus (HIV) infection contributes heavily to HIV-related functional disability (1). This HIV-related fatigue may be promoted by the regulatory immune peptides (cytokines) which are increased in HIV infection and have been shown to be somnogenic in both clinical trials and animal models (2–8). These peptides induce slow-wave sleep (sleep stages 3 and 4). Of particular interest is the monokine (monocyte/macrophage peptide) tumor necrosis factor α (TNF-α). Rabbits intravenously injected with recombinant human TNF-α showed significant dose-dependent increases in slow-wave sleep (9). TNF-α is increased (up to 300 pg/ml) in the sera of persons with HIV infection (10–13), including children (14, 15), and cells from HIV-infected subjects produce increased amounts (1.5–6 times control levels) of TNF-α (16–18). A review of the role of TNF-α in acquired immune deficiency syndrome (AIDS) concluded that the increases in serum TNF-α are closely correlated with the severity of the disease, and it was suggested that TNF-α antagonists be used therapeutically in AIDS (19). The regulatory mechanisms governing the increases in TNF-α and its relationship to sleep modulation and to the fatigue seen in HIV infection remain unclear.

For patients with HIV infection spanning the range of severity compared with healthy HIV-seronegative persons, fatigue was significantly more a problem, interfered more with daily activities, and interfered with work, employment, and driving (1). The HIV-positive subjects were significantly more likely to be unemployed, to feel fatigued through more hours of the day, to sleep more, to nap more, and to have more diminished midmorning alertness. We have further documented pathological sleepiness in HIV patients (20), observing a significant relationship between poor sleep and absolute $CD4^+$ cell number, and in which most of the variance was accounted for by daytime dysfunction and nighttime sleep disturbances (21). These data demonstrate that fatigue and sleep disturbances contribute to HIV-related morbidity and disability and may contribute to the mild neurocognitive deficit described in early HIV infection. The HIV-related fatigue is most severe in patients with advanced disease, and it worsens disability due to HIV-1-associated cognitive/motor complex (AIDS-related "dementia"). Little is known about the physiology or etiology of this fatigue or its relationship to changes in nocturnal sleep in HIV infection (22–24). This area was recently reviewed (25). Slow-wave sleep is increased in early, asymptomatic HIV infection (22–25) and decreases back to normal percentages but with loss of the normal cyclic rhythmicity in advanced, symptomatic HIV infection (AIDS-related complex and AIDS) (25–27).

TNF-α (cachectin) is a 17-kDa polypeptide monokine (157 aa) that induces a range of systemic neurological, metabolic, hematological, and endocrinological changes. TNF-α is probably involved in the physiologic regulation of sleep (9). When TNF-α is used clinically for cancer chemotherapy, central nervous system (CNS) toxicity is an important dose-limiting factor. Fatigue is prominent among the CNS effects caused by TNF-α (5–8). The origin of the effects of TNF-α on sleep could be either systemic or within the CNS. Even in situations with increased serum TNF-α and progressive encephalopathy, cerebrospinal fluid TNF-α is not increased (14, 15, 28). Thus, peptides of this size would seem to have poor access to the brain, limited by the blood–brain barrier, yet they seem to elicit central effects. Signals across the blood–brain barrier initiated by cytokines may be common, however. TNF-α specifically may influence the CNS through areas of access of the peripheral circulation to the CNS which bypass the blood–brain barrier—e.g., organum vasculosum of the lamina terminalis, median eminence, or area postrema

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Abbreviations: AZT, 3′-azido-3′-deoxythymidine; CNS, central nervous system; EEG, electroencephalogram; HIV, human immunodeficiency virus; QEEG, quantitative electroencephalographic; TNF, tumor necrosis factor.

(28–30). Further, the barrier may be more permeable in infection, when the entry of TNF-α may be enhanced by inflammation and a consequent local or global compromise of the barrier—e.g., migrating macrophages (5–8). Finally, specific active transport mechanisms have been proposed for human interleukin 1α, interleukin 1β, and TNF-α (31, 32).

With quantitative electroencephalographic (QEEG) technology, brain activity can be reliably analyzed by using spectral power estimates (in microvolts squared) or the related parameter, the square root of power, the spectral amplitude estimates (in microvolts) at each frequency. We subjected the sleep electroencephalograms (EEG) of HIV-positive and control subjects to QEEG analysis, examining delta amplitude.

MATERIALS AND METHODS

We studied eight HIV-positive men and two HIV-seronegative men, all age 22–42 (Tables 1 and 2). Our inability to include more than two control subjects is discussed below. All subjects were in the San Diego HIV Neurobehavioral Research Center (HNRC) cohort, and the eight HIV-positive subjects and one control were Navy participants in the HNRC. Dates of HIV infection were estimated for all HIV-positive subjects as the center of the time interval between the last negative and first positive HIV antibody test. Subjects were excluded if they had active intracranial neoplasms, active *Pneumocystis carinii* pneumonia, other severe active opportunistic infections, or any severe medical illness. The subjects were 1993 Centers for Disease Control and Prevention category A1, A2, B1, or B2. Staging was done by the HNRC Medical Core. $CD4^+$ lymphocyte counts were performed on each subject by cytofluorometry. The normal range, as referenced by our pathology laboratories, is 400–1400 cells per μl. Three of the HIV subjects had $CD4^+$ cell counts of $\geq$400 cells per μl (see Table 1 and Fig. 4). Five of the HIV subjects had counts of <400 cells per μl (Table 1 and Fig. 4). In the two control subjects, zero was used as the time since seroconversion. Six of the eight HIV positive patients were receiving AZT. Subjects were free of psychoactive medications (e.g., hypnotics, sedatives, antidepressants) for at least 2 weeks before inclusion in the study, with the exception of two HIV-positive subjects who occasionally used the tranquilizer triazolam (Halcion, Upjohn). Subject 8 used ibuprofen for headache and back pain, which is of note, given its antiinflammatory action. We excluded subjects diagnosed as having a current DSM-III-R Axis I psychiatric disorder (33), as diagnosed by the Psychiatry Core (based on Diagnostic Interview Schedule interview) of the HNRC.

Subjects spent three consecutive nights in the sleep laboratory: an adaptation, a baseline, and a study night. On the first night, we recorded standard EEG, electromyogram, and electrooculogram montages; oximeter readings; thoracic and abdominal respiratory movement; electrocardiogram; and tibialis electromyogram, to screen for any primary nocturnal sleep disorders. All physiologic signals were digitized and sampled at 272 samples per second. EEG channels were referenced to linked ears (EEG electrodes A1–A2). On the baseline- and study-night recordings, only EEG, electrooculogram, and chin electromyogram were used to permit sleep-stage and QEEG analyses of accommodated subjects. Actual sleep time for subjects was 8 hr. The protocol sidereal time for the sleep period was 2200 to 0600. Actual lights-out time varied from 2200 to 2315. When lights-out was later than 2200, an extension of the sleep period was allowed to permit an 8-hr sleep period.

First, all nocturnal polygraph recordings were hand scored on the basis of 30-sec epochs. Examination of night 2 and 3 sleep records showed minimal effect of phlebotomy on sleep. Then QEEG analysis was performed on each digitized sleep record, using digital period/amplitude analysis. For the delta frequency band (0.5–4.0 Hz), the average root-mean-square (rms) was calculated in microvolts for each 30-sec epoch. The amplitude measures derived from EEG electrode positions C3(A1–A2) were used, giving quantitative estimates of power (spectral amplitude estimates) for each epoch. Epochs displaying any movement artifact were excluded from QEEG analysis; eye movements were not separately addressed. The personnel scoring the records by either method could not be kept blinded to subject category and medical health. The accuracy and precision of rms analysis for QEEG was confirmed by comparison with fast Fourier transform analysis (34).

Blood sampling during sleep was conducted on the third night. Thirty minutes before lights-out, an intravenous catheter was inserted. Phlebotomy was performed without disturbing the subject. Beginning at lights-out, blood samples were obtained every 15 min for the first 3 hr of sleep, then again every 15 min for the final 2 hr of sleep. Total phlebotomy volume was 161 ml drawn across 23 sampling times. Blood samples were drawn into ice-chilled Vacutainers that were returned to the ice bath immediately. Samples were then spun in a refrigerated centrifuge at 4°C to separate the plasma. Plasma samples were aliquoted into iced 0.5-ml cryovials and stored at −80°C until assay. Time from blood draw to storage at −80°C was <20 min for all samples. This care with keeping the samples cold is needed to prevent loss of detectable TNF-α. Plasma from the first nine subjects (eight HIV and one control) was assayed for human TNF-α by enzyme-linked immunosorbent assay (ELISA) with a sensitivity of 12 pg/ml. This Factor-Test assay from Genzyme became permanently unavailable. Plasma from the other control subject was assayed by immunoradiometric assay (IRMA) from Medgenix,

Table 1. Delta-frequency sleep and TNF-α in the eight HIV-positive subjects and two control subjects

Subject	*r*	$CD4^+$ cells, no./μl	Time since seroconversion, year(s)	Mean delta, μV	Mean TNF-α, pg/ml	Mean % delta	Mean % delta 3rd 1/3rd	AZT* use
1	0.42	320	0.2	26.00	74.05	3.5	5.1	No
2	0.32	845	Control	19.25	49.95	1.3	0.9	No
3	−0.51	308	5.1	29.02	13.00	15.4	18.2	Yes
4	0.22	260	2.5	22.35	55.91	2.5	1.0	Yes
5	0.56	550	0.08	28.11	75.33	10.4	14.1	No
6	0.68	420	3.7	32.30	44.56	15.0	15.1	Yes
7	−0.05	100	3.3	18.11	42.55	2.7	0.0	No
8	0.30	240	4.9	25.68	72.83	9.8	5.8	Yes
9	0.47	525	4.0	27.62	49.78	14.5	9.9	Yes
10	0.19	1020	Control	28.60	13.13	18.5	13.1	No

r, Pearson product–moment correlation coefficient from the cross-correlation analysis; mean delta, mean delta amplitude by QEEG analysis through the night's sleep; mean TNF-α, mean of the TNF-α values drawn through the night sleep (the TNFα level for control subject no. 10 is not comparable to the rest; see text); mean % delta, mean percent delta-frequency sleep across the whole night on the visually scored sleep record; mean % delta 3rd 1/3, mean percent delta-frequency sleep during the final one-third of the sleep period on the visually scored sleep record.
*3′-Azido-3′-deoxythymidine.

Table 2. Clinical descriptions of the subjects

Subject	Age, years	Marital status	Race	Employment	Clinical description
1	42	Separated	Caucasian	Full time	Daytime fatigue, sleep problems, otherwise well, no substance abuse history. Two months earlier with seroconversion had malaise, diaphoresis, and disorientation.
2	36	Divorced	Hispanic	Full time	Medically and mentally well, no substance abuse history.
3	38	Never married	Caucasian	Full time	Daytime fatigue, 90-min naps, no sleep complaints, frequent headaches, muscle pain, back pain, skin rashes, and inhalant allergies. Mentally well, no substance abuse history.
4	22	Never married	Asian	Full time	Daytime fatigue, no sleep complaints. Oral thrush, persistent diarrhea. Mentally well but alcohol abuse history.
5	29	Separated	Caucasian	Full time	Severe daytime fatigue, sleep-onset difficulty, poor sleep quality, tired on awakening. Medically and mentally well, no substance abuse history.
6	38	Never married	Caucasian	Unemployed and homeless	Occasional sleep-onset difficulty. Medically well. Diagnosed with adjustment disorder with depressed mood. History of alcohol and cocaine dependence and suicide attempts.
7	36	Never married	Hispanic	Unemployed	Poor sleep quality, severe sleep-onset difficulty for two years. Problematic physical tiredness, denied fatigue. Night diaphoresis, oral thrush, and nausea. Mentally well, no substance abuse history.
8	39	Never married	Caucasian	Full time	Poor sleep quality for 1 year, occasional sleep-onset difficulty. Frequent headaches, muscle pain, back pain, oral herpes simplex, seropositive for history of hepatitis A and B, inhalant allergies. Ibuprofen, a nonsteroidal antiinflammatory medication, used for headaches, muscle, and back pain. Mentally well, no substance abuse history.
9	28	Never married	Caucasian	Full time	Daytime fatigue, poor subjective sleep quality with sleep-onset difficulty for 2 years. Oral thrush, oral herpes simplex. Mentally well, no substance abuse history.
10	27	Never married	Hispanic	Full time	No fatigue or sleep complaints. Medically and mentally well, no substance abuse history.

with a sensitivity of 5 pg/ml. The intraassay coefficient of variation is 2.2–6.0%, and the interassay coefficient of variation is 2.8–7.0%. While we recognize the concern of having one control evaluated with a different methodology, we considered it necessary to have at least two controls, rather than one, for comparison. Changing availability of and sensitivity of immune assays prevents us from testing further control subjects with this same methodology. An entirely new series of patients and controls will need to be tested to replicate this work. ELISA/IRMA results were confirmed by subjecting a subset of the samples to TNF bioassay using the standard L929 cell line. TNF bioassay specifically of TNF-α was accomplished by incubation of the bioassay cultures with excess neutralizing antibody to TNF-β. All TNF-α assays were run blind to the subject category or severity of illness.

For TNF-α concentrations, spline curves were fit by interpolation to the sample values. Cross-correlation plots were then constructed between the QEEG delta frequency amplitude and TNF-α with SYSTAT version 5.0 (1990–1992; Systat, Evanston, IL), and Pearson correlation coefficients (r values) were calculated between the plasma TNF-α (pg/ml) and delta amplitude (μV) curves. Coupling in this context was defined as a Pearson r of >0.25 between curves. Correlations between these initial correlation coefficients and time since seroconversion to HIV-positive status (as one measure of duration of HIV infection) were calculated by using Spearman ρ rank-order correlation coefficients.

RESULTS

In all subjects we found a previously unrecognized nocturnal cyclic variation in plasma TNF-α levels. In 6 of the 10 subjects (1 control subject, 3 HIV-seropositive patients with $CD4^+$ number of >400 cells per μl, and two HIV-positive patients with $CD4^+$ number of <400 cells per μl), we further found a coupling ($r > 0.25$ between curves) between plasma levels of TNF-α and sleep EEG delta frequency spectral amplitude (Figs. 1 and 2). This coupling was not present in the remaining 4 subjects. Three HIV-positive subjects with $CD4^+$ number of <400 cells per μl and 1 control subject had poor correlation between TNF-α and delta amplitude through the night's sleep (Fig. 3), despite relative medical health (none of the HIV-positive subjects had progressed to full AIDS). Figs. 1–3 are representative plots of data of high and low r.

Estimated time since seroconversion to HIV-seropositive status was used as a measure of duration of HIV infection (Table 1). When days since the subjects seroconverted (independent variable) was plotted against the previously calculated correlation coefficients between TNF-α and delta amplitude (dependent variable) for the five subjects with <400 $CD4^+$ cells per μl, we observed a significant negative correlation: Spearman $\rho = -0.64, n = 5, P < 0.05$. The longer the duration of HIV infection, the poorer the correlation between TNF-α and delta amplitude in these subjects. This relationship was not significant in the 5 subjects with >400 $CD4^+$ cells per μl, in the

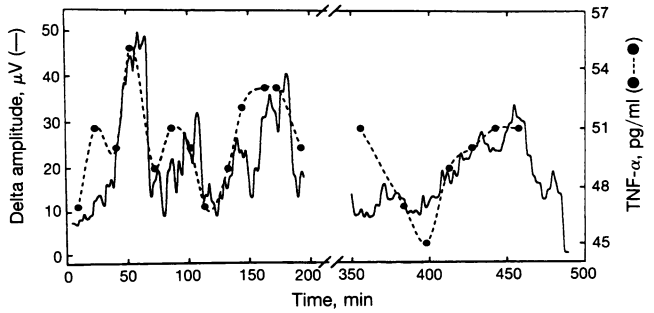

FIG. 1. Plot of data from a control subject, subject no. 2, showing EEG delta-frequency spectral amplitude estimates in microvolts (continuous line and left axis) and plasma levels of TNF-α in picograms per milliliter (connected discrete sample times and right axis) through the night's sleep (480 min = 8 hr). Note the cyclic changes with a period of 90–120 minutes in TNF-α and delta amplitude that rise and fall in concert (coupling; Pearson $r = 0.35$). TNF-α points were connected by a mathematically fit spline curve.

group of all 8 HIV-positive subjects (Fig. 4), or in the group of all 10 subjects. For the group of all 8 HIV-positive subjects, $\rho = -0.38$, $P = 0.4$; for the group of all 10 subjects, $\rho = -0.28$, $P = 0.3$.

DISCUSSION

Increased levels of TNF-α mRNA have been detected in the frontal subcortical white matter of brains of demented HIV-infected patients compared with both nondemented HIV-infected patients and healthy control subjects (35, 36). It is possible that elevated brain levels of TNF-α in advanced HIV infection interfere with a normal slow-wave sleep control mechanism involving peripheral TNF-α. Increased peripheral TNF-α production could also contribute to the hypersomnia and fatigue of HIV infection by anomalous access to the brain with resultant impairment. The present study supports these concepts by demonstrating a coupling between blood TNF-α and sleep EEG delta-frequency amplitude that is less often seen in subjects with lower $CD4^+$ cell count and longer duration of HIV illness.

Six of the eight HIV-seropositive subjects were receiving AZT. Four studies have shown that AZT has no significant effect on the sleep of HIV-infected individuals (26, 37, 38). It is unknown whether AZT affects plasma TNF-α concentration. The small number of controls in the present study was obligatory because of changing Navy research priorities, making the Naval Sleep Research Facility unavailable for study of further subjects, and changing availability of, and sensitivity of,

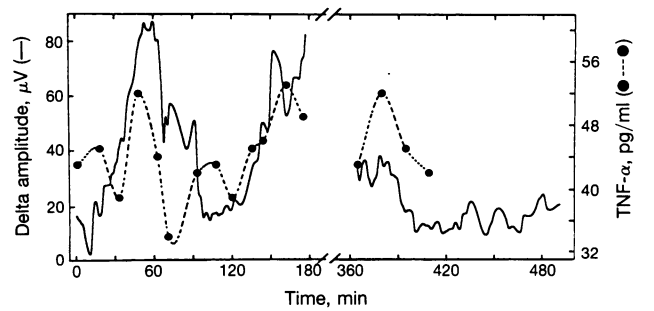

FIG. 2. Plot of data from the HIV patient with $CD4^+$ count of 420 cells per μl, HIV-positive for 3.7 years, subject no. 6. The pattern is similar to the control subject's pattern (Fig. 1), with cyclic changes with the same periodicity in TNF-α and delta amplitude (Pearson $r = 0.53$). Coupling of the data is similar for the other two patients with $CD4^+$ count ≥400 cells/μl. The mean values of TNF-α and delta amplitude vary (see text). Points raised in the legend to Fig. 1 apply.

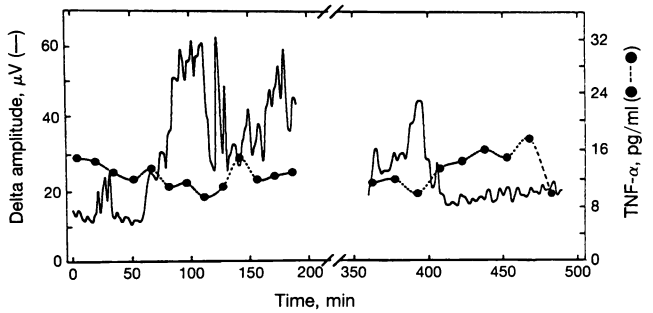

FIG. 3. Plot of data from the HIV patient with $CD4^+$ cell count of 240 cells per μl, HIV-positive 5.1 years, subject no. 3. The coupling is lost in the pattern of cyclic changes in TNF-α and delta amplitude. The loss of coupling between TNF-α and delta amplitude is again similar for the other four patients with $CD4^+$ count <400 cells per μl (Pearson $r = -0.51$). The mean values of TNF-α and delta amplitude vary (see Tables 1 and 3 and text).

assay methodology for TNF-α. The second control (subject 10) was studied in a different, newly built sleep research facility at Scripps, and his plasma was assayed for TNF-α by a different method. The control subjects were matched for age and gender.

Subjects 8 and 10 do not fit a paradigm of lower correlation between TNF-α and delta amplitude in subjects with low $CD4^+$ cell number and longer duration of HIV infection. The other four HIV-positive subjects with $r > 0.25$ have either short duration of illness (subject 1), normal $CD4^+$ cell number (subjects 6 and 9), or both (subject 5). The three HIV-positive subjects with $r < 0.25$ all have low $CD4^+$ number and longer duration of illness (subjects 3, 4, and 7). Subject 10 was studied in a different sleep laboratory facility, and his TNF-α level was assayed with a different methodology than the other nine subjects. Subject 8 was taking ibuprofen, a nonsteroidal anti-inflammatory agent (Table 2). It is possible that the ibuprofen use by subject 8 affected the physiological coordination between TNF-α and delta amplitude in a way that improved the correlation between these two variables. If this speculation is adopted for the purpose of discussion, and it is further recognized that subject 10 is a poor comparison subject in at least two ways, we are left with the possibility that the physiologic coordination of interest (between TNF-α and delta amplitude) varies predictably with $CD4^+$ cell number and duration of HIV infection. However, we note that the ibuprofen does not seem to have lowered subject 8's mean TNF-α level, which is the third highest among the subjects, at 72.83 pg/ml (Table 1). Therefore, these data cannot be used to address the question of the use of antiinflammatory medications to decrease HIV-associated CNS dysfunction.

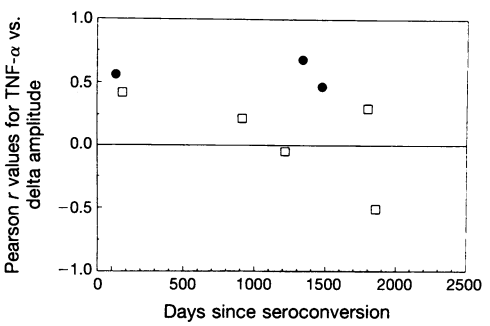

FIG. 4. Plot of the time since HIV seroconversion to seropositive status (independent variable on x axis) vs. the correlation coefficients between TNF-α and delta-frequency amplitude (dependent variable on y axis) for the eight HIV-seropositive subjects. The subjects with normal $CD4^+$ lymphocyte number (>400 cells per μl) (●) and low $CD4^+$ cell number (<400 cells per μl) (□) are indicated.

Table 3. Comparisons of TNF-α and delta amplitude among the three groups

	Mean	SD	SEM
HIV$^+$ with <400 CD4$^+$ cells per μl (n = 5)			
TNF-α, pg/ml	56.56	16.47	9.51
Delta amplitude, μV	29.34	2.57	1.49
HIV$^+$ with >400 CD4$^+$ cells per μl (n = 3)			
TNF-α, pg/ml	51.67	25.22	11.28
Delta amplitude, μV	24.23	4.16	1.86
Control (n = 2)			
TNF-α, pg/ml	31.54	26.04	18.41
Delta amplitude, μV	23.92	6.61	4.68

Mean TNF-α levels and mean delta amplitude were statistically similar among groups in this limited sample ($P > 0.05$ for all comparisons); see *Discussion*.

As seen in Tables 1 and 3, mean TNF-α levels and delta voltage were variable within groups and similar among groups in this limited sample. In a larger cohort of subjects studied at our institute without QEEG measures, mean TNF-α through the night (unpublished work) and slow-way sleep in the second half of the night (39) were higher in HIV-positive subjects than in control subjects, in agreement with published reports (5–8), and these two measures were highly correlated (unpublished work).

It is important to conceptualize that delta power or amplitude, a parameter quantitated by spectral analysis of the EEG, is a different variable than slow-wave sleep stages 3 or 4, also called delta sleep, which is clinically determined by visual scoring of sleep records. In the subjects studied, all stages of non-rapid-eye-movement and rapid-eye-movement sleep had >5–10 μM of delta-frequency spectral amplitude associated with them. Delta amplitude seemed to follow TNF-α better than non-rapid-eye-movement stages 3 and 4.

The literature introduces the concept of an influence of HIV infection on the hypothalamic–pituitary–adrenal (HPA)-axis hormones and of a possible effect of cortisol on acute-phase-response peptides such as interleukin 1β and TNF-α. The findings in these areas have been inconsistent, making it difficult for definitive conclusions to be reached. In contrast, the diurnal variation in the mammalian HPA-axis hormones is well established. It is possible that circadian-linked endocrine factors have confounded the TNF–EEG relationships examined in this study.

Percentage and time of delta sleep are two of the classically examined sleep parameters. Visual scoring of sleep stages indicates that delta sleep is increased in early, asymptomatic HIV infection (22–25), but this increase is lost in advanced HIV infection, along with the rest of normal cyclical sleep structure (25–27). In other populations decreases or interruptions in slow-wave sleep are associated with fatigue. The use of QEEG analysis may resolve this question. Recording and analysis of the amplitude in the delta frequencies provide a valuable index of CNS activity (40–44).

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